

Supplemental Selenium Source in Holstein Steers Challenged with Intranasal Bovine Infectious Rhinotracheitis Virus: Blood Metabolites, Hormones, and Cytokines

T. L. Covey,^{*1} N. E. Elam,[†] PAS, J. A. Carroll,[‡] D. B. Wester,[§] M. A. Ballou,^{*} D. M. Hallford,[#] and M. L. Galyean,^{*} PAS

^{*}Department of Animal and Food Sciences, Texas Tech University, Lubbock 79409; [†]Clayton Livestock Research Center, New Mexico State University, Clayton 88415; [‡]Livestock Issues Research Unit, USDA-ARS, Lubbock, TX 79403; [§]Department of Natural Resources Management, Texas Tech University, Lubbock 79409; and [#]Department of Animal and Range Sciences, New Mexico State University, Las Cruces 88001

ABSTRACT

As an integral constituent of glutathione peroxidase (GSH-Px), Se is a vital antioxidant during stress. Calves are exposed to multiple factors that can lead to oxidative stress, including viral infection. Our objective was to evaluate the effects of supplementation of Se and Se source on immune function of Holstein steer calves challenged with infectious bovine rhinotracheitis virus (IBRV). Twenty-four Holstein steers (initial BW = 170 ± 0.6 kg) were assigned randomly to 3 treatments: 1) no supplemental Se (control); 2) 1 mg/steer daily of Se from Se yeast (Sel-Plex, Alltech Inc., Nicholasville, KY); or 3) 5 mg/steer daily of Se from sodium selenite. Treatments were

fed for 35 d before intranasal inoculation with IBRV (d 0) and were continued through 21 d after the challenge. Whole blood Se was greater ($P < 0.001$) in steers receiving Sel-Plex than in the other 2 groups, but GSH-Px activity ($P > 0.80$) was not affected by treatment. Serum interleukin-1 β concentrations tended to be greater ($P = 0.09$) in steers fed selenite compared with control steers, and tumor necrosis factor- α concentrations 12 h after the challenge tended ($P = 0.09$) to be greater in steers fed Sel-Plex compared with control steers. Steers in the selenite group had decreased ($P \leq 0.02$) serum urea N at 4 and 72 h after the challenge compared with control steers, and steers receiving Sel-Plex had increased serum urea N at 72 h ($P = 0.008$) after the IBRV challenge compared with control steers. Serum glucose concentrations tended ($P = 0.06$) to be

less in steers that received the selenite treatment versus no supplemental Se. Serum concentrations of nonesterified fatty acids ($P > 0.40$), insulin, cortisol, prolactin, and triiodothyronine were not affected ($P \geq 0.24$) by treatment. The present data help to define the immune response to a viral challenge in cattle. Additional research is needed to clarify the role of Sel-Plex Se in the immune response of cattle to viral infections.

INTRODUCTION

Phagocytosis, an early immune defense mechanism in viral infections, is accompanied by a respiratory burst that results in the production of reactive oxygen species (ROS) or free radicals. Various antioxidant enzymes and molecules catalyze the deactivation of ROS (Sies,

¹Corresponding author: tanyacoveyphd@gmail.com

2007). When antioxidant activities are decreased, particularly during pathogenic stimulation of phagocytic leukocytes (Arthington et al., 1996), ROS can accumulate. Oxidative stress occurs when the generation of ROS exceeds the capacity of antioxidant mechanisms (Chirase et al., 2004). Stress accompanying marketing and shipping cattle has been associated with decreased disease resistance, potentially leading to oxidative stress (Chirase et al., 2004), which could decrease antioxidant capacity and increase lipid peroxidation in the body, thereby increasing susceptibility to disease (Sheridan et al., 2007). Several micronutrients, vitamins, and trace minerals have crucial roles in primary antioxidant systems (Sies, 2007). Thus, understanding the role of nutrients that are involved in the antioxidant defense system should aid in the development of management strategies to combat bovine respiratory disease (BRD) in beef cattle.

Selenium is an integral component of glutathione peroxidase (GSH-Px; Rotruck et al., 1973), a family of enzymes that protect cells from oxidative damage (Rotruck et al., 1973; Lawrence et al., 1974), thereby protecting cell membranes from damage (Sies, 2007). In addition to GSH-Px, Se could mediate immune function and overall health through its role in viral pathogenesis. Increased pathogenicity of viruses has been reported with oxidative stress, in which non-pathogenic strains of certain viruses mutated to become pathogenic in Se-deficient individuals (Beck et al., 1994, 1995; Nelson et al., 2001).

Selenium supplementation often increases plasma GSH-Px, which is considered an effective measure of Se status. Effects of Se on animal performance and immune function have been equivocal, however, with supplemental Se resulting in variable and inconsistent responses (Duff and Galyean, 2007). Given these conflicting results, the objective of the present experiment was to evaluate the role of Se supplementation and Se source on immune response to a viral challenge in Holstein calves. Because infectious

Table 1. Treatment averages of whole blood Se and glutathione peroxidase (GSH-Px), and serum malondialdehyde (MDA) of Holstein steers given either no supplemental Se or Se from either an organic complex (Sel-Plex) or sodium selenite and challenged intranasally with infectious bovine rhinotracheitis virus (IBRV)

Item ¹	Treatment ²			SE ³	Contrast ⁴	
	Control	Sel-Plex	Selenite		C1	C2
Se, ng/mL	253.1	276.1	261.2	3.57	<0.001	0.12
GSH-Px, ⁵ units/g	148.1	146.8	149.4	5.75	0.88	0.86
MDA, nmol/mL	25.2	24.4	23.0	2.15	0.79	0.48

¹Se, GSH-Px, and hemoglobin were measured in whole blood collected on d -28, -14, 0, 1, 3, 7, 14, and 21, whereas MDA was measured in serum collected on d -3, 0, 1, and 7 relative to the IBRV challenge. No treatment × day of study interactions were detected, $P \geq 0.28$.

²Control = no supplemental Se; Sel-Plex = 1 mg/steer daily of Se from Sel-Plex Se (Alltech Inc., Nicholasville, KY); selenite = 5 mg/steer daily of Se from sodium selenite.

³SE of treatment means, $n = 8$ steers/treatment. All values are adjusted for baseline covariates.

⁴P-value for contrasts. C1 = control versus Sel-Plex; C2 = control versus selenite.

⁵One unit is defined as the amount of enzyme that will cause the oxidation of 1.0 nmol nicotinamide adenine dinucleotide phosphate per min at 25°C. Values are expressed per gram of hemoglobin.

bovine rhinotracheitis virus (IBRV) is widely acknowledged as a primary causative factor in the pathogenesis of BRD feedlot cattle, we used an IBRV

challenge model to assess immune system responses to treatments.

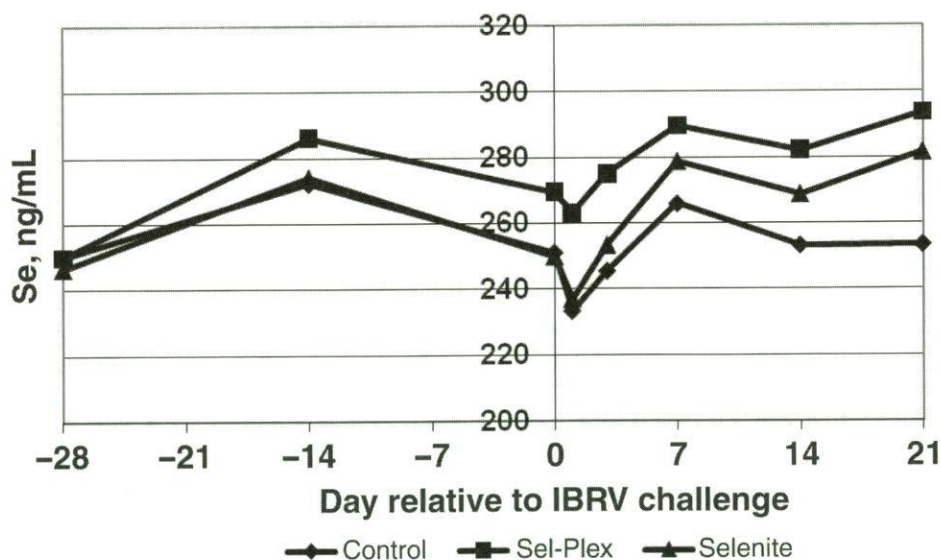


Figure 1. Whole blood Se concentrations in Holstein steers given either no supplemental Se or Se from either an organic complex (Sel-Plex, Alltech Inc., Nicholasville, KY) or sodium selenite and challenged intranasally with infectious bovine rhinotracheitis virus (IBRV). Pooled SE of treatment means ranged from 6.46 to 6.47 over sampling times. No treatment × day of study interactions were detected ($P = 0.52$). All values were adjusted for baseline covariates.

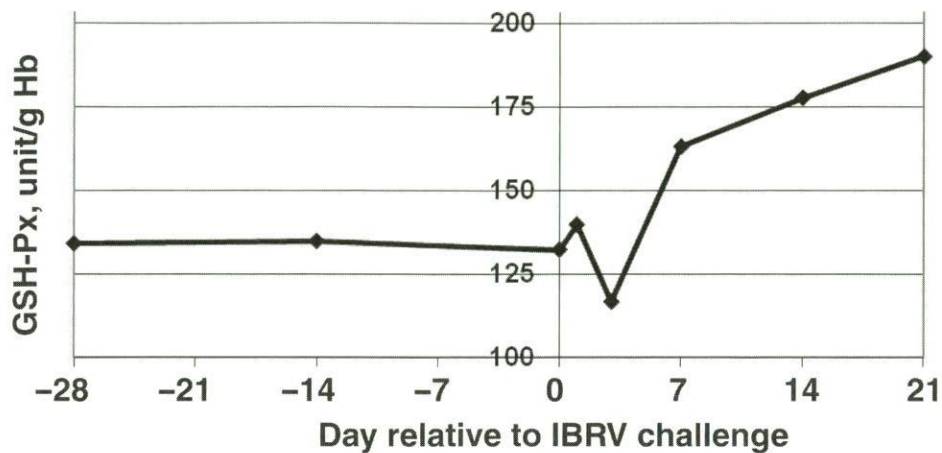


Figure 2. Whole blood glutathione peroxidase (GSH-Px), averaged across treatments and adjusted for baseline covariates, in Holstein steers given either no supplemental Se or Se from either an organic complex (Sel-Plex, Alltech Inc., Nicholasville, KY) or sodium selenite and challenged intranasally with infectious bovine rhinotracheitis virus (IBRV). The pooled SE was 6.12 across sampling times. No treatment \times day of study interactions were detected ($P = 0.28$). One unit of GSH-Px is defined as the amount of enzyme that will cause the oxidation of 1.0 nmol nicotinamide adenine dinucleotide phosphate per min at 25°C. The GSH-Px values are expressed per gram of hemoglobin.

MATERIALS AND METHODS

All procedures involving animals were reviewed and approved by the Institutional Animal Care and Use Committees at Texas Tech University.

Animals

Thirty Holstein steers (BW = 149.7 ± 3.42 kg) were received at the Texas Tech University Burnett Center research feedlot on March 30, 2007, after a 2-h transit from Clovis, New Mexico. Complete details of animal management practices, diet formulation and analyses, treatment assignment, feeding, weighing, sampling, and IBRV challenge procedures are found in Covey et al. (2009). The steers were assigned randomly to 1 of 3 treatments (8 steers/treatment): 1) no supplemental Se (control); 2) supplemental Se supplied in the form of a Se-yeast product; (0.2% Se premix; Sel-Plex, Alltech Biotechnology Inc., Nicholasville, KY); or 3) supplemental Se supplied in the form of sodium selenite (selenite; 0.2% Se premix; Animal Science Products Inc., Nacogdoches, TX). Treatments were designed to supply 0, 1, and 1 mg/d, respectively, of supplemental Se to each steer; however, as indicated in

Covey et al. (2009), analysis after the completion of the experiment of the top dress containing sodium selenite and the 0.2% selenite premix from which the top dress was prepared revealed a manufacturing error in which the source was labeled and marketed as containing 0.20% Se but actually contained 1.0% Se. Thus, the dose of supplemental Se for the selenite treatment was 5.0 mg/d.

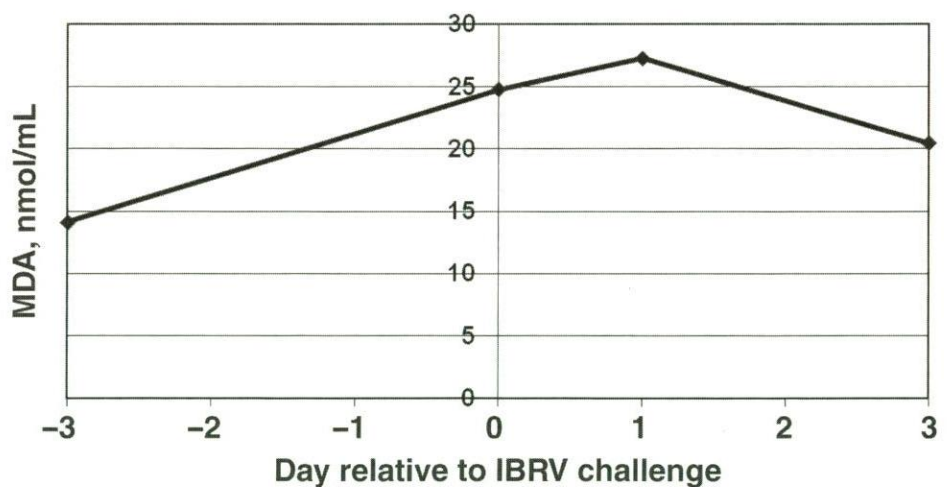


Figure 3. Serum malondialdehyde (MDA), averaged across treatments and adjusted for baseline covariates, in Holstein steers given either no supplemental Se or Se from either an organic complex (Sel-Plex, Alltech Inc., Nicholasville, KY) or sodium selenite and challenged intranasally with infectious bovine rhinotracheitis virus (IBRV). Pooled SE ranged from 1.02 to 2.93 across sampling times. No treatment \times day of study interactions were detected ($P = 0.47$).

Serum and Blood Analyses

Details of blood collection times are provided in Covey et al. (2009). Briefly, blood was collected via jugular venipuncture (Vacutainer SST and Vacutainer EDTA, BD, Franklin Lakes, NJ) on d -35, -28, -21, -14, -7, -3, 7, 14, and 21 relative to the IBRV challenge. Jugular catheters were used for intensive blood collection periods. While jugular catheters were in place and patent (d 0; 0 h = immediately before administration of virus, and 4, 8, 12, and 24 h, and 3 d after the challenge), blood was drawn from the catheter line. As described in Covey et al. (2009), whole blood was transported at room temperature, and each sample was evaluated within 2 h of collection for differential cell counts using the Cell-Dyn System 3700 (Abbott Laboratories, Abbott Park, IL). Remaining whole blood samples were stored frozen until analyzed for whole blood Se concentrations by the Michigan State University Diagnostic Center for Population and Animal Health (Lansing, MI). Briefly, to assay Se concentration in whole blood, 200 μ L of each sample was diluted 1:20 with a solution containing 0.5% (wt/vol) EDTA and Triton X-100, 1% (vol/vol) ammonia hydroxide, 2% (vol/vol) propanol, and 20 μ g/kg

of scandium, rhodium, indium, and bismuth as internal standards. All samples were analyzed on an Agilent 7500ce ionized coupled plasma mass spectrometer (Agilent Technologies Inc., Santa Clara, CA).

A commercial kit was used to measure GSH-Px activity in whole blood (No. 703102, Cayman Chemical, Ann Arbor, MI), which was measured at 340 nm in a Benchmark microplate reader (Bio-Rad Laboratories, Hercules, CA). Intraassay CV was 7.8%.

Serum was separated by centrifugation ($1,500 \times g$ for 20 min at 4°C) and was then poured into plastic, screw-top containers as described in Covey et al. (2009) and stored frozen. Serum concentrations of interleukin-1 β (IL-1 β), interleukin-2 (IL-2), tumor necrosis factor- α (TNF- α), interferon- γ (IFN- γ), interleukin-4 (IL-4), and interleukin-6 (IL-6) were determined by use of a custom multiplex (2-plex for IL-1 β and IL-2 and 4-plex for IL-4, IL-6, TNF- α , and IFN- γ) ELISA validated for bovine cytokines (Endogen SearchLight Plus CCD Imaging System, Pierce Biotechnology Inc., Woburn, MA). Intraassay CV for IL-1 β , IL-2, IL-6, TNF- α , and IFN- γ were 8.9, 8.9, 8.6, 9.6, and 5.3%, respectively. Although IL-4 was measured in all serum samples, too many samples were below the detection limits of the standard curve for the assay used; therefore, IL-4 data were not analyzed statistically.

Malondialdehyde (MDA) was measured in serum with a thiobarbituric acid reactive substances assay kit (No. 0801192; Zeptometrix Corp., Buffalo, NY). Intra- and interassay CV for MDA were 3.6 and 4.6%, respectively. Serum glucose concentrations were determined by a manual colorimetric assay with orthotoluidine reagent as described by Webster et al. (1971), with absorbance read at 540 nm (Beckman DU-50 spectrophotometer, Beckman Instruments Inc., Fullerton, CA). Intra- and interassay CV for the glucose assay were 1.4 and 9.0%, respectively. Serum urea-N concentration was determined using the colorimetric diacetylmonoxime assay (manual method) at 540 nm as

described by Wybenga et al. (1971). Intra- and interassay CV for the assay were 2.0 and 3.2%, respectively.

Serum nonesterified fatty acids (NEFA) concentrations were analyzed colorimetrically with a commercially available kit (Wako USA, Richmond, VA) as described by Ballou et al. (2009). Absorbance was measured at 550 nm in the Benchmark visual light spectrophotometer-microplate reader described previously for the GSH-Px assay. The intraassay CV for the NEFA assay was 4.9%.

Serum concentrations of cortisol, triiodothyronine (T3), and insulin were determined by solid-phase RIA using components of commercial kits (Coat-A-Count, Siemens Diagnostic,

Los Angeles, CA). The cortisol, T3, and insulin assays were validated as described by Kiyama et al. (2004), Wells et al. (2003), and Reimers et al. (1982), respectively; within- and between-assay CV were less than 10%. Serum prolactin concentrations were determined by double antibody RIA as described by Spoon and Hallford (1989), using primary antisera and purified standard and iodination preparations supplied by the National Hormone and Peptide Program (Harbor-UCLA Medical Center, Torrance, CA). Serum prolactin was quantified in a single assay with a CV of 11%.

Table 2. Treatment averages of serum interleukin-1 β (IL-1 β), interleukin-2 (IL-2), interleukin-6 (IL-6), interferon- γ (IFN- γ), and tumor necrosis factor- α (TNF- α) concentrations of Holstein steers given either no supplemental Se or Se from either an organic complex (Sel-Plex) or sodium selenite and challenged intranasally with infectious bovine rhinotracheitis virus (IBRV)

Item ¹	Treatment ²				Contrast ⁴	
	Control	Sel-Plex	Selenite	SE ³	C1	C2
IL-1 β , pg/mL	228.3	201.8	292.5	26.35	0.48	0.09
IL-2, pg/mL	132.4	1,201.5	549.9	1,058.64	0.47	0.77
IL-6, pg/mL	77.0	55.3	40.69	26.18	0.56	0.32
IFN- γ , pg/mL	218.7	225.0	216.5	5.40	0.42	0.78
TNF- α , ⁵ pg/mL						
0 h	10.5	24.7	22.9	5.13	0.06	0.10
4 h	1.8	22.2	8.4	10.57	0.16	0.64
8 h	15.9	14.8	16.7	4.96	0.28	0.21
12 h	8.7	22.4	9.8	4.60	0.09	0.49
24 h	26.2	39.8	16.6	25.96	0.14	0.56
72 h	43.4	27.1	17.2	12.91	0.11	0.34

¹IL-1 β , IL-2, IL-6, and IFN- γ were measured in serum collected at 0, 4, 8, 12, 24, and 72 h relative to the IBRV challenge. At 4 h, IL-2 values were below the assay detection limits; therefore, IL-2 treatment averages do not include the 4-h sample. No treatment \times sampling time interactions were detected for IL-1 β , IL-2, IL-6, or IFN- γ ($P \geq 0.24$).

²Control = no supplemental Se; Sel-Plex = 1 mg/steer daily of Se from Sel-Plex Se (Alltech Inc., Nicholasville, KY); selenite = 5 mg/steer daily of Se from sodium selenite.

³SE of treatment means, $n = 8$ steers/treatment. All values are adjusted for baseline covariates.

⁴ P -value for contrasts. C1 = control versus Sel-Plex; C2 = control versus selenite.

⁵TNF- α was measured in serum collected at 0, 4, 8, 12, 24, and 72 h relative to the IBRV challenge. For these times, a treatment \times sampling time interaction was detected, $P = 0.07$.

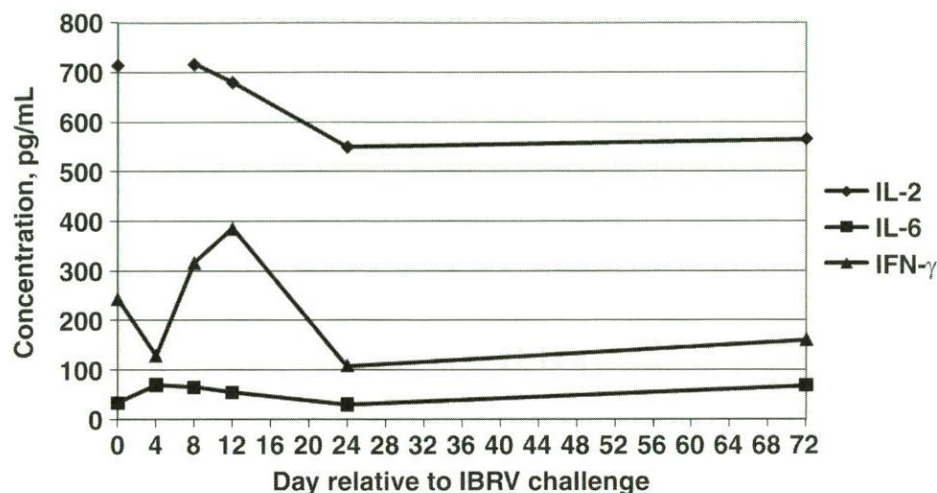


Figure 4. Serum interleukin-2 (IL-2), interleukin-6 (IL-6), and interferon- γ (IFN- γ) concentrations, averaged across treatments and adjusted for baseline covariates, in Holstein steers given either no supplemental Se or Se from either an organic complex (Sel-Plex, Alltech Inc., Nicholasville, KY) or sodium selenite and challenged intranasally with infectious bovine rhinotracheitis virus (IBRV). Pooled SE ranged from 427.13 to 969.42, 4.88 to 32.83, and 72.24 to 100.69 for IL-2, IL-6, and IFN- γ , respectively, across sampling times. No treatment \times day of study interactions were detected ($P \geq 0.41$).

Statistical Analyses

All data were analyzed as a completely random design with repeated measures in time using the MIXED procedure (SAS Institute Inc., Cary, NC). The appropriate covariance structure for each variable was determined by comparing the Akaike and Schwarz Bayesian criteria from the MIXED printout for compound symmetry, autoregressive (type 1), and antedependence structures and selecting the structure that resulted in the minimal values for these criteria. Data collected before steers were placed in the individual stanchions were analyzed with pen as the experimental unit (2 pens/treatment), whereas all data collected after the steers entered the barn (d -7 relative to challenge) and after the challenge were analyzed with steer as the experimental unit (8 steers/treatment). For all variables, the respective baseline value for each steer was included in the model as a covariate and retained in the model regardless of significance. Because of the error associated with the Se concentration of the selenite treatment, the 2 Se treatments were not compared. Thus, comparisons (determined using the PDIF option in SAS when

the treatment or treatment \times sampling time interaction was significant, $P \leq 0.05$) were limited to control versus Sel-Plex and control versus selenite. When the sampling time \times treatment interaction was significant ($P \leq 0.05$), contrasts were evaluated within sampling times.

RESULTS AND DISCUSSION

Whole Blood Selenium, Glutathione Peroxidase Activity, and Serum Malondialdehyde Concentration

There were no treatment \times sampling day interactions ($P \geq 0.28$) for whole blood Se concentrations, GSH-Px activity (expressed per gram of hemoglobin), serum MDA, or hemoglobin concentrations (Table 1).

Table 3. Treatment averages of glucose, nonesterified fatty acids (NEFA), and urea N in serum of Holstein steers given either no supplemental Se or Se from either an organic complex (Sel-Plex) or sodium selenite and challenged intranasally with infectious bovine rhinotracheitis virus (IBRV)

Item ¹	Treatment ²			SE ³	Contrast ⁴	
	Control	Sel-Plex	Selenite		C1	C2
Glucose, mg/dL	111.1	107.3	101.3	3.393	0.24	0.06
NEFA, μ Eq/L	530.2	482.4	526.2	41.093	0.43	0.95
Urea N, ⁵ mg/dL						
0 h	22.9	23.9	23.1	1.21	0.57	0.89
4 h	24.7	25.8	20.6	0.68	0.27	<0.001
8 h	19.6	21.5	19.1	1.70	0.44	0.84
12 h	18.3	20.5	18.1	1.57	0.33	0.93
24 h	20.8	21.1	18.2	1.01	0.85	0.08
72 h	21.7	11.5	12.5	2.45	0.008	0.02

¹Glucose and NEFA were measured in serum collected at 0, 4, 8, 12, 24, and 72 h relative to the IBRV challenge. No treatment \times day of study interactions were detected for glucose or NEFA ($P \geq 0.36$).

²Control = no supplemental Se; Sel-Plex = 1 mg/steer daily of Se from Sel-Plex Se (Alltech Inc., Nicholasville, KY); selenite = 5 mg/steer daily of Se from sodium selenite.

³SE of treatment means, $n = 8$ steers/treatment. All values are adjusted for baseline covariates.

⁴ P -value for contrasts. C1 = control versus Sel-Plex; C2 = control versus selenite.

⁵Urea N was measured in serum collected at 0, 4, 8, 12, 24, and 72 h relative to the IBRV challenge. For these times, a treatment \times day interaction was detected, $P = 0.09$.

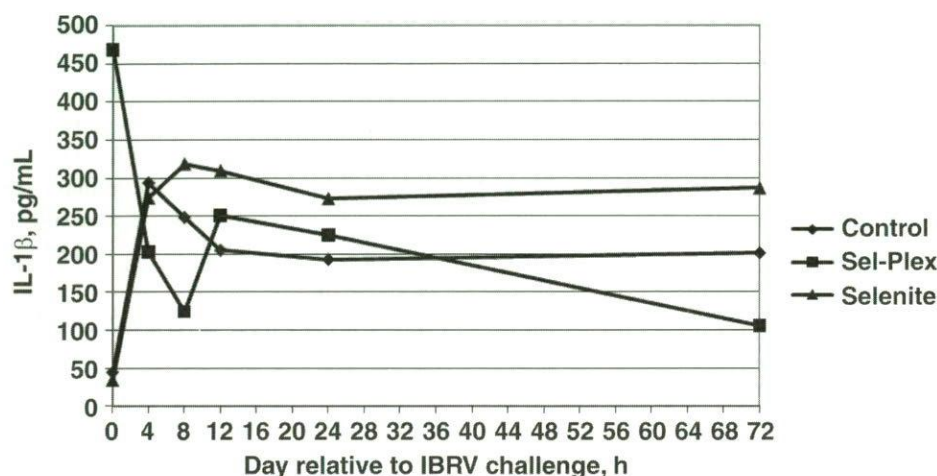


Figure 5. Serum serum interleukin-1 β (IL-1 β) concentrations in Holstein steers given either no supplemental Se or Se from either an organic complex (Sel-Plex, Alltech Inc., Nicholasville, KY) or sodium selenite and challenged intranasally with infectious bovine rhinotracheitis virus (IBRV). Pooled SE of treatment means ranged from 27.61 to 77.23 over sampling times. No treatment \times day of study interactions were detected ($P = 0.24$). All values were adjusted for baseline covariates.

Whole blood Se concentrations were greater ($P < 0.001$) when Sel-Plex was fed than in control steers (Figure 1). Although the Sel-Plex versus selenite comparison was confounded with Se dose, it is noteworthy that the whole blood Se concentration was greater with the lower dose of Sel-Plex than the higher dose of selenite. This finding is consistent with previous research comparing Sel-Plex with sodium selenite (Pehrson et al., 1999) and suggests that even with approximately 5 times the Se intake, Sel-Plex resulted in greater whole blood Se concentrations than selenite. According to the limits described by Counotte and Hartmans (1989) and Koller et al. (1983) for deficient, low, marginal, and adequate Se status classifications, all 3 treatment groups were adequate in terms of their whole blood Se concentrations. These results were not unexpected, as the basal diet used in the experiment had an analyzed Se concentration of 0.41 mg/kg of DM, which exceeds the concentration suggested by NRC (1996) as adequate to meet Se requirements.

Surprisingly, given the differences in whole blood Se concentrations, whole blood GSH-Px activity did not differ among treatments ($P > 0.80$), which presumably reflects the adequate Se concentration of the basal

diet. Nonetheless, GSH-Px activity increased substantially in response to the IBRV challenge (Figure 2), providing evidence (similar to that noted for rectal temperature; see Covey et al., 2009) of a successful challenge to the immune system of the steers as a result of applying the IBRV challenge model. Similar to GSH-Px activity, serum MDA concentrations, an index of oxidative stress, did not differ ($P \geq 0.48$) among treatments, again presumably reflecting the adequacy

of both Se and vitamin E in the basal diet. Chirase et al. (2004) reported increased MDA concentrations in serum from steers in response to transport stress and BRD. In their experiment, MDA was measured in serum 3 d before and 1 d after transit (3,105 km; 19.2 h). Chirase et al. (2004) found that on the day after transit, steers that subsequently required at least 3 treatments for BRD or that died from the disease had 200 and 43% greater ($P < 0.01$) serum MDA concentrations, respectively, than healthy steers (no BRD treatments). This difference was evident as early as 3 d before transit, with steers that would eventually die from BRD having 144% greater MDA concentrations than those that never required treatment for BRD (Chirase et al., 2004). In the current experiment, serum MDA averaged over the 3 treatments (Figure 3) exhibited a 94% increase from d -3 through d 1 before slightly decreasing; however, serum MDA on d 7 was still 45% greater than on d -3 relative to the IBRV challenge.

Cytokines and Serum NEFA Concentrations

Serum cytokine and NEFA concentrations are reported in Tables 2 and 3, respectively. No treatment \times

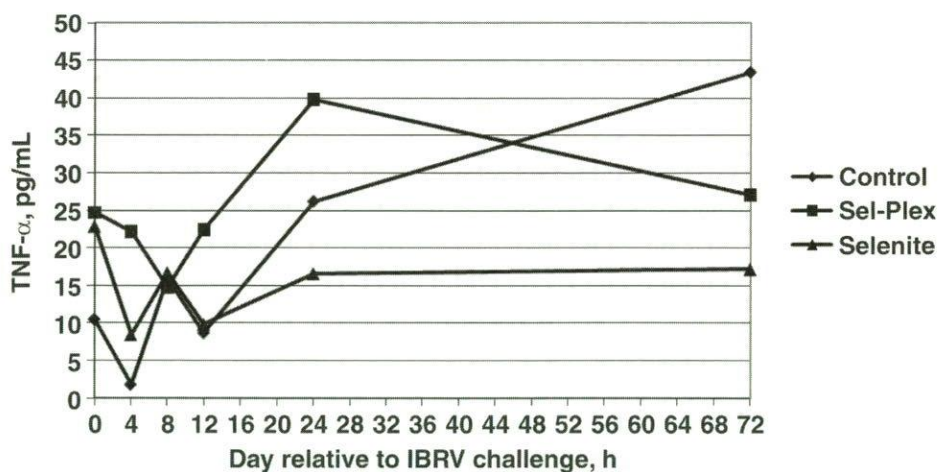


Figure 6. Serum tumor necrosis factor- α (TNF- α) concentrations in Holstein steers given either no supplemental Se or Se from either an organic complex (Sel-Plex, Alltech Inc., Nicholasville, KY) or sodium selenite and challenged intranasally with infectious bovine rhinotracheitis virus (IBRV). Pooled SE of treatment means ranged from 4.28 to 25.96 over sampling times. A treatment \times day of study interaction was detected ($P = 0.04$). All values were adjusted for baseline covariates.

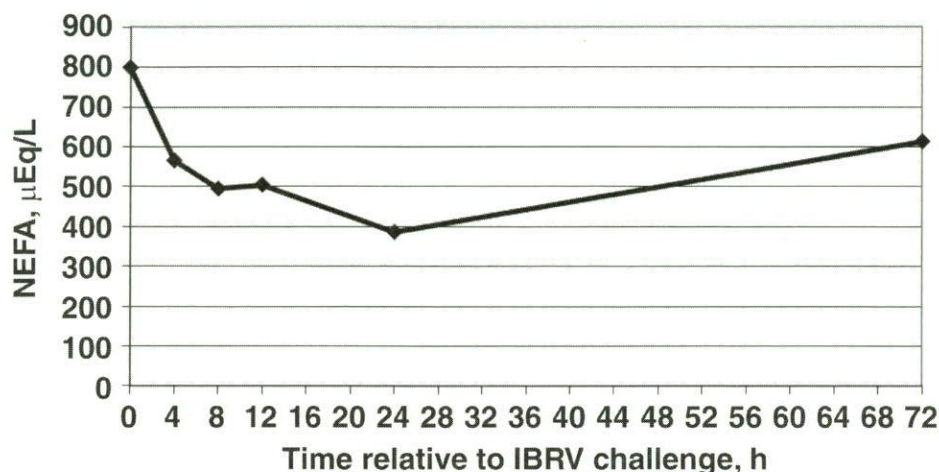


Figure 7. Serum nonesterified fatty acid (NEFA) concentrations, averaged across treatments and adjusted for baseline covariates, in Holstein steers given either no supplemental Se or Se from either an organic complex (Sel-Plex, Alltech Inc., Nicholasville, KY) or sodium selenite and challenged intranasally with infectious bovine rhinotracheitis virus (IBRV). Pooled SE ranged from 26.35 to 55.54 across sampling times. No treatment \times day of study interactions were detected ($P = 0.36$).

sampling time interactions ($P > 0.24$) or treatment effects were detected for serum concentrations of the cytokines IL-2, IL-6, or IFN- γ ($P > 0.30$; Figure 4). For serum IL-1 β (Figure 5), however, steers in the selenite treatment group tended ($P = 0.09$) to have greater concentrations (averaged over sampling times; no treatment \times sampling time interaction, $P > 0.24$) than control steers (292.5 pg/mL vs. 228.3 pg/mL). Activated monocytes and macrophages secrete IL-1 β , which acts to facilitate inflammation during pathogen insult (Goldsby et al., 2003). Specifically, IL-1 β promotes fever by stimulating the thermoregulatory center of the hypothalamus, increases vascular permeability to allow extravasation of cells to the infection site, and activates T and B lymphocytes. It is not clear what promoted the differences in serum IL-1 β among treatment groups, especially because there were no differences between treatment groups for any blood cell count data (Covey et al., 2009). Nonetheless, the rectal temperature data reflected a febrile response to increases in IL-1 β in all groups after the IBRV challenge (Covey et al., 2009).

A treatment \times time interaction ($P = 0.07$) was detected for serum concentrations of TNF- α (Table 2). By the beginning of the IBRV chal-

lenge (at 0 h), blood from steers that received supplemental Se from either Sel-Plex or sodium selenite tended to have greater ($P \leq 0.10$) TNF- α concentrations than steers that did not receive supplemental Se. At 12 h after the challenge, Sel-Plex steers tended ($P = 0.09$) to have an increased (13.7 ng/mL) serum TNF- α concentration compared with control steers. Kushibiki et al. (2002) found a close, positive association between TNF- α and serum NEFA concentra-

tions, indicating a rapid and sustained increase in NEFA after a single injection of recombinant bovine TNF- α in nonpregnant Holstein heifers. In the present experiment, both serum TNF- α and NEFA decreased initially in response to the IBRV challenge in the steers receiving selenite and Sel-Plex, with TNF- α beginning an upward trend in all treatment groups between 8 (Sel-Plex) and 12 h (control and selenite) after the challenge (Figure 6). After the initial decrease, serum NEFA concentrations did not begin to increase until approximately 12 h after the increase in TNF- α (24 h after the challenge; Figure 7). Even so, no differences among treatments were detected in NEFA concentrations ($P \geq 0.43$).

Serum Urea Nitrogen and Glucose Concentrations

A treatment \times time interaction ($P = 0.09$; Table 3) was detected for serum urea N concentration, with the Sel-Plex treatment increasing ($P < 0.001$) serum urea N at 4 h after the challenge compared with the control treatment (Figure 8). In addition, at 24 h after the challenge, steers that received the control treatment tended ($P = 0.08$) to have a greater serum urea N concentration than steers

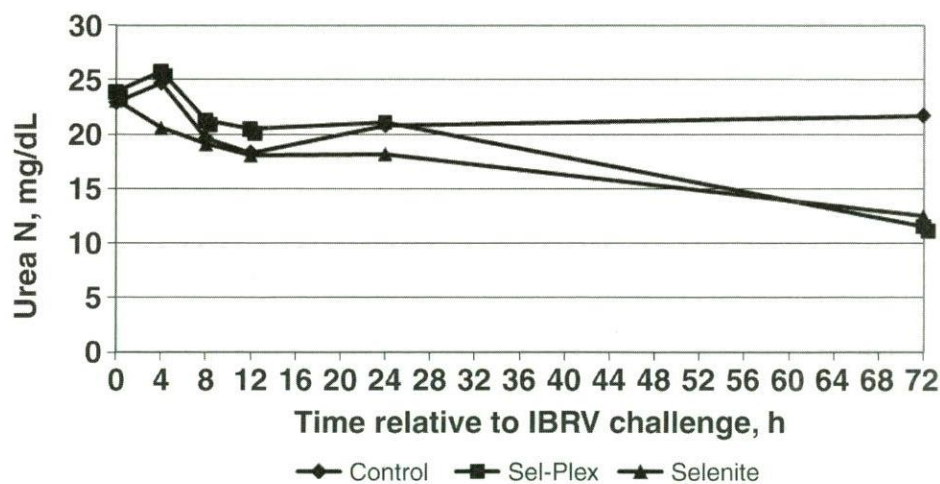


Figure 8. Serum urea N concentrations in Holstein steers given either no supplemental Se or Se from either an organic complex (Sel-Plex, Alltech Inc., Nicholasville, KY) or sodium selenite and challenged intranasally with infectious bovine rhinotracheitis virus (IBRV). Pooled SE of treatment means ranged from 0.68 to 2.45 over sampling times. A treatment \times day of study interaction was detected ($P = 0.0$). All values were adjusted for baseline covariates.

Table 4. Treatment averages of insulin, cortisol, prolactin, and triiodothyronine (T3) in serum of Holstein steers given either no supplemental Se or Se from either an organic complex (Sel-Plex) or sodium selenite and challenged intranasally with infectious bovine rhinotracheitis virus (IBRV)

Item ¹	Treatment ²				Contrast ⁴	
	Control	Sel-Plex	Selenite	SE ³	C1	C2
Insulin, ng/mL	0.93	0.99	0.99	0.098	0.63	0.68
Cortisol, ng/mL	18.5	19.8	15.6	1.71	0.60	0.24
Prolactin, ng/mL	72.4	84.0	90.8	14.77	0.59	0.39
T3, ng/mL	1.35	1.26	1.23	0.104	0.56	0.42

¹Insulin and cortisol were measured in serum collected on d -7, at 0, 4, 8, 12, 24, 72 h, and at 7, 14, and 21 d relative to the IBRV challenge. Prolactin and T3 were measured in serum collected on d -7, 0, 7, 14, and 21 relative to challenge. No treatment \times day interactions were detected, $P \geq 0.16$.

²Control = no supplemental Se; Sel-Plex = 1 mg/steer daily of Se from Sel-Plex Se (Alltech Inc., Nicholasville, KY); selenite = 5 mg/steer daily of Se from sodium selenite.

³SE of treatment means, $n = 8$ steers/treatment. All values are adjusted for baseline covariates.

⁴ P -value for contrasts. C1 = control versus Sel-Plex; C2 = control versus selenite.

receiving selenite. By 72 h after the IBRV challenge, however, steers in the both the Sel-Plex and selenite groups had serum urea N concentrations that were 9.7 and 8.7 mg/dL less ($P \leq 0.02$), respectively, than those of control steers. Serum glucose concentrations were not affected by a treatment \times sampling time interaction ($P > 0.91$), and did not differ between the control and Sel-Plex treatments; however, the average glucose concentra-

tion of the selenite group tended ($P = 0.06$) to be less than concentrations in control steers (Table 3).

Serum Hormone Concentrations

Treatment averages for insulin, prolactin, cortisol, and T3 are presented in Table 4. No treatment \times sampling time interactions ($P > 0.15$) were detected, nor were there differ-

ences among treatments for serum insulin, prolactin, or T3 ($P \geq 0.39$). A clear relationship between glucose and insulin can be observed in Figures 9 and 10. In addition, an interesting response of both variables to the IBRV challenge was noted, in which serum insulin concentration increased rapidly, peaking at 8 to 12 h after the challenge and stabilizing by 24 h after the challenge. Serum glucose decreased rapidly within 4 h of the challenge and continued declining gradually; however, it is noteworthy that serum glucose never returned to baseline (173.7 mg/dL) by the last sampling time (72 h; 92.7 mg/dL) after the IBRV challenge.

A treatment \times sampling time interaction was not detected ($P > 0.15$) for cortisol, and serum cortisol did not differ ($P > 0.60$) among treatments. Glucocorticoids such as cortisol can lead to increased serum glucose concentrations as a result of increased gluconeogenesis and decreased glucose utilization (Brockman, 1986). Besides a marked decrease from d -7 to 0, and an increase from d 3 to 7, serum cortisol seemed relatively stable in the present experiment (Figure 11). It is important to note the events to which serum cortisol concentrations responded. In particular, spikes in serum cortisol concentrations occurred at the sampling times when the steers were relocated, whether into or out of the stall barn. No increases in cortisol were noted in response to the IBRV challenge or at any of the blood collection times when the jugular catheters were used for blood collection. This is an important finding because it shows that the catheters were a successful means of collecting blood from animals without altering their stress profile. It is not known, however, to what extent, if any, serum cortisol concentrations responded to the application of the rectal temperature probes and jugular vein catheters initially or when they were removed because serum from these times was not analyzed for cortisol.

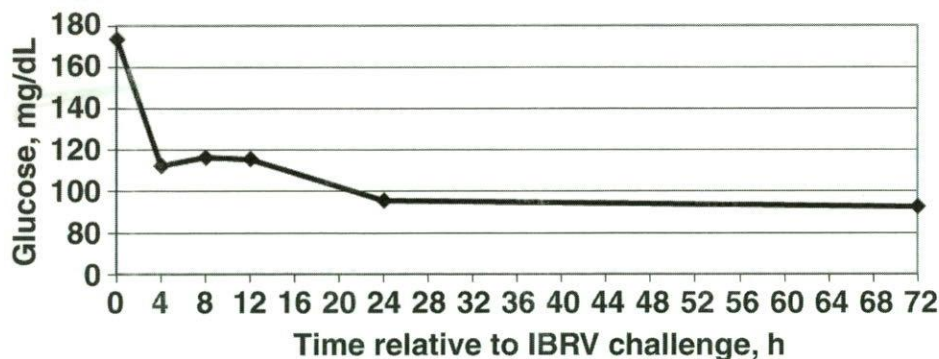


Figure 9. Serum glucose concentrations, averaged across treatments and adjusted for baseline covariates, in Holstein steers given either no supplemental Se or Se from either an organic complex (Sel-Plex, Alltech Inc., Nicholasville, KY) or sodium selenite and challenged intranasally with infectious bovine rhinotracheitis virus (IBRV). Pooled SE ranged from 1.93 to 7.42 across sampling times. No treatment \times day of study interactions were detected ($P = 0.91$).

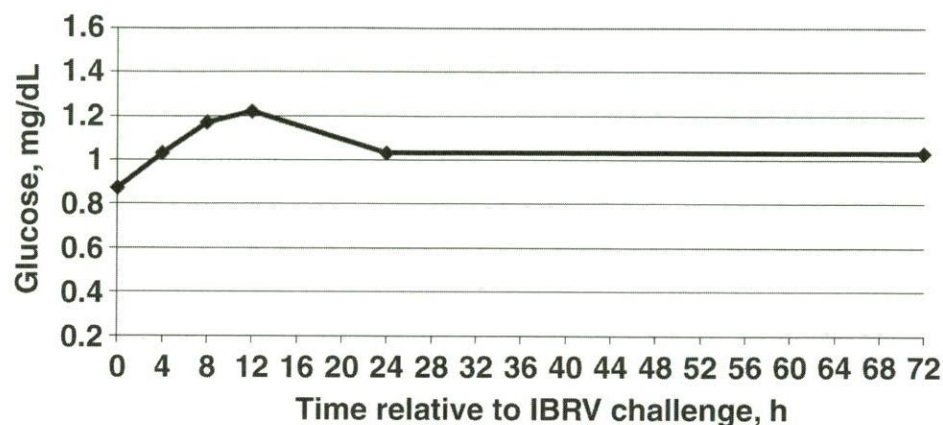


Figure 10. Serum insulin concentrations, averaged across treatments and adjusted for baseline covariates, in Holstein steers given either no supplemental Se or Se from either an organic complex (Sel-Plex, Alltech Inc., Nicholasville, KY) or sodium selenite and challenged intranasally with infectious bovine rhinotracheitis virus (IBRV). Pooled SE ranged from 0.05 to 0.14 across sampling times. No treatment \times day of study interactions were detected ($P = 0.16$).

IMPLICATIONS

In Holstein steers fed a basal diet with adequate Se, supplying supplemental Se from 2 different sources did not seem to greatly influence the metabolic and immune responses associated with an IBRV challenge. The interrelationships between and among the variables measured are intriguing, and although the present experiment did not yield profound treatment differences, the subtle treatment differences in proinflammatory cytokines and some metabolites (such as serum urea N concentration) warrant further

research. Specifically, evaluation of the cellular, immunological, and metabolic responses to viral infections should lead to a greater understanding of the potential role of Se in diets of immunologically stressed cattle, including identification of the dose and duration of Se supplementation required to alter immune status favorably and prevent or ameliorate BRD in stressed cattle. Moreover, based on changes in whole blood Se concentrations, further research should be conducted to closely evaluate the value of the increased Se absorption when Se

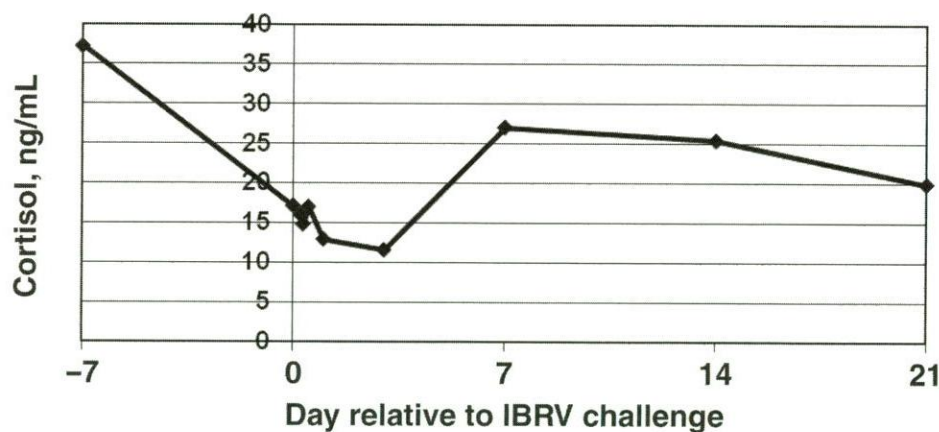


Figure 11. Serum cortisol concentrations, averaged across treatments and adjusted for baseline covariates, in Holstein steers given either no supplemental Se or Se from either an organic complex (Sel-Plex, Alltech Inc., Nicholasville, KY) or sodium selenite and challenged intranasally with infectious bovine rhinotracheitis virus (IBRV). The pooled SE was 2.36 across sampling times. No treatment \times day of study interactions were detected ($P = 0.99$).

is provided as Sel-Plex as opposed to selenite.

ACKNOWLEDGMENTS

The authors appreciate the financial support of this research and the graduate program of T. L. Covey through a grant from Alltech Inc. (Nicholasville, KY). The authors also express appreciation to DSM Nutritional Products (Parsippany, NJ), Elanco Animal Health (Indianapolis, IN), Fort Dodge Animal Health (Overland Park, KS), Intervet (Millsboro, DE), and Kemin Industries (Des Moines, IA) for supplying various products used during the experiment. Further gratitude is extended to A. F. Parlow (National Hormone and Peptide Program, Harbor-UCLA Medical Center, Torrance, CA) for materials used in the prolactin RIA. Outstanding technical support in animal care and feed milling was provided by K. Robinson and R. Rocha (Texas Tech University Burnett Center).

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